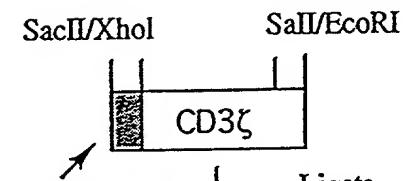
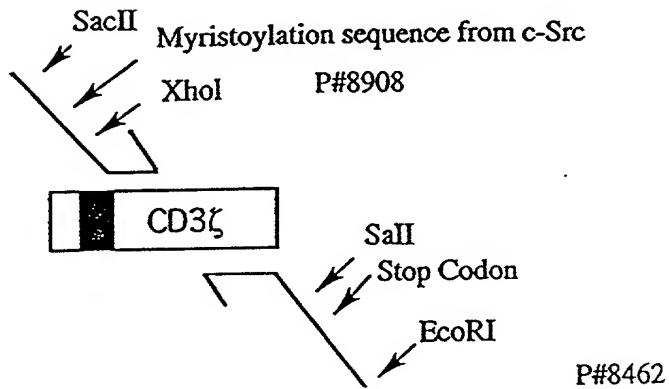


Figure 1/21

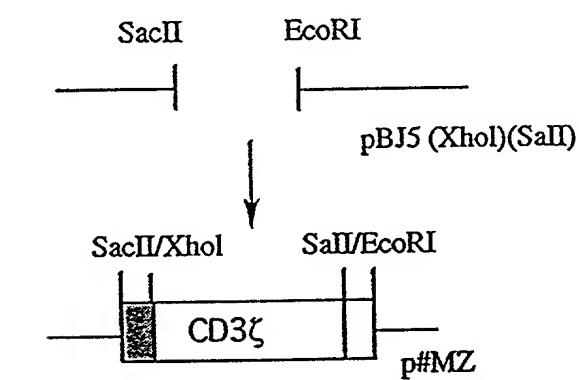
Construction of intracellular signalling chimera:

1. PCR myristoylated CD3 ζ



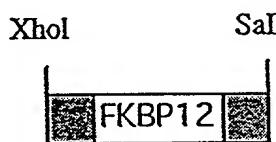
2. Cut and clone PCR fragment

*The MZE series contains a 9aa HA epitope at the 3' end.



3. SEQUENCE insert

4. Cut at Xhol or SalI and add FKBP domains



From plasmid #FK12/KS

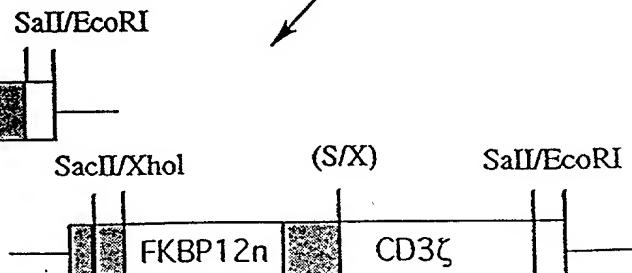
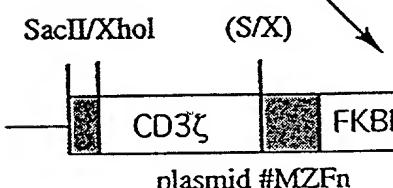
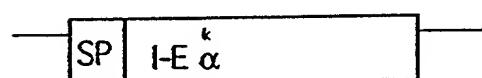


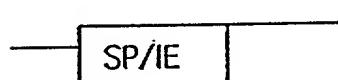
Figure 2/21

Construction of extracellular signaling chimera:

1. PCR murine signal peptide

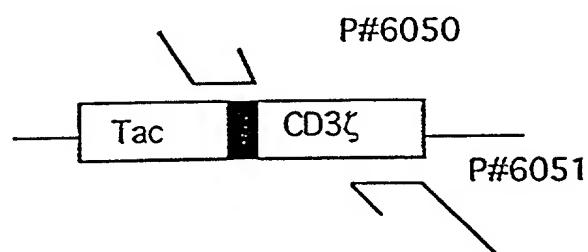


P#6048

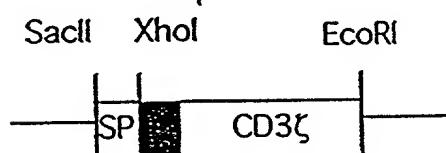
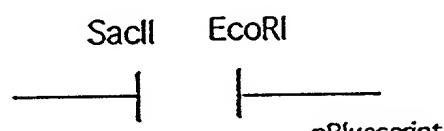
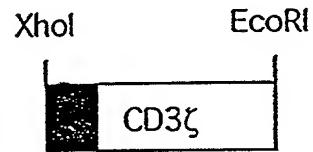
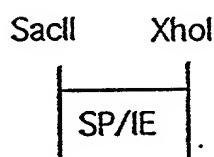


P#6049

2. PCR CD3 trans-membrane and cytoplasmic domains



T ζ



plasmid #SPZ/KS
SEQUENCE insert*

Cut Xhol

Figure 3A/21

3. PCR FKBP12

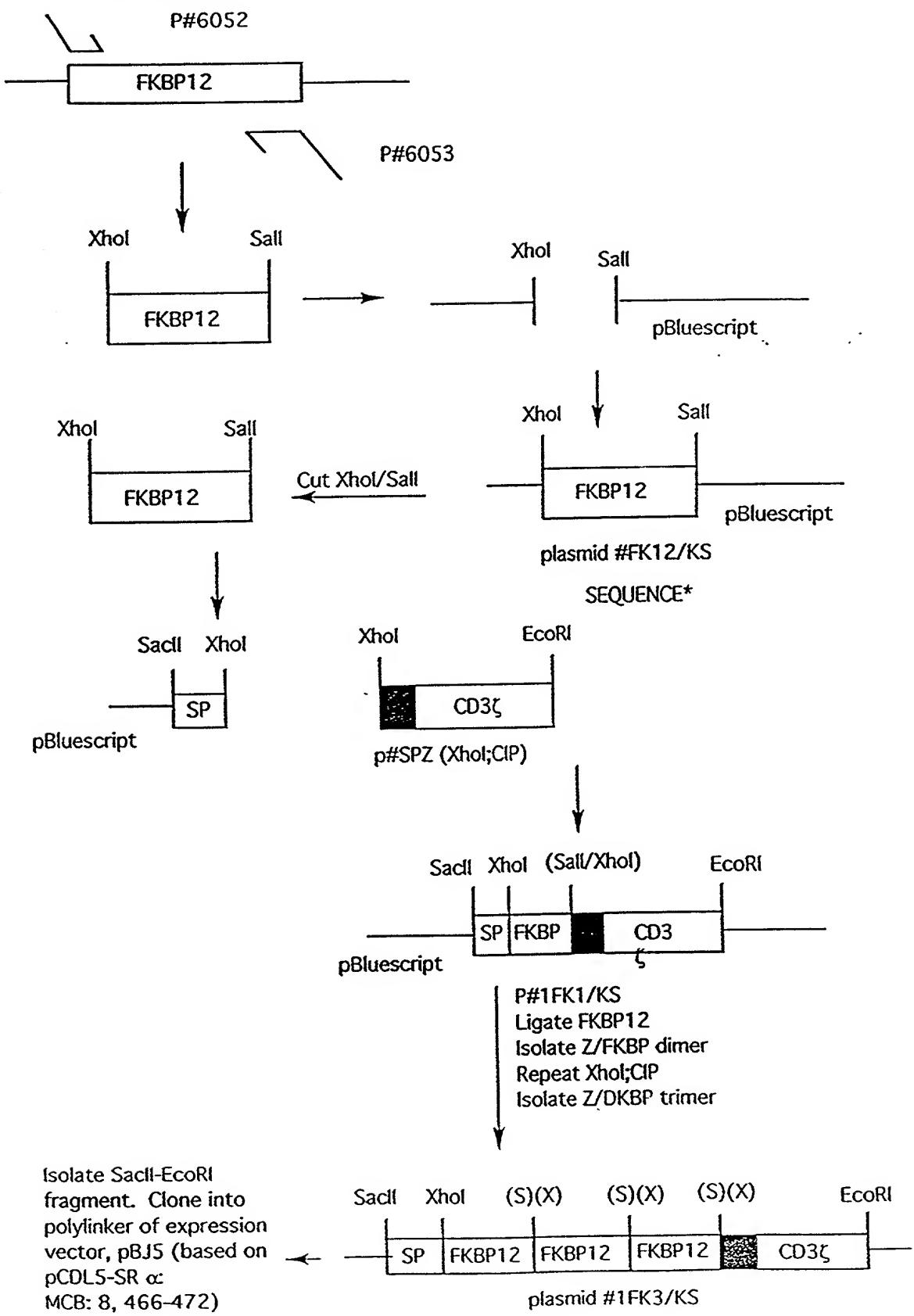
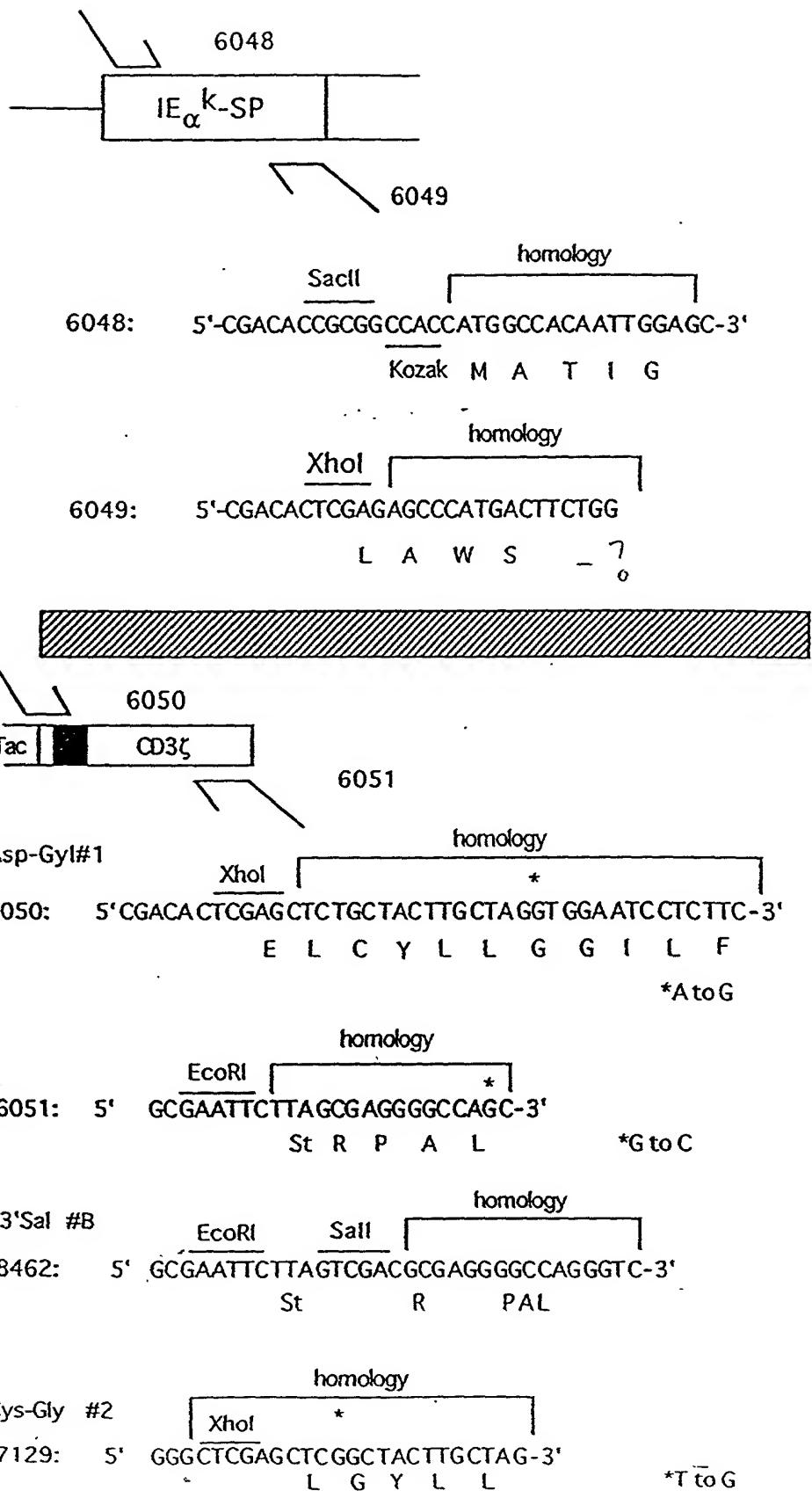


Figure 3B/21



CYCC

6568: 5'-CGACACTCGAGGTGACGGACAAGGTC-3'

homology

Xhol

6569: 5'-CGACAGTCGACCCAATCAGGGACCTC-3'

homology

Sall

EPITOPE

7850: 5'-TCGAGTATCCGTACGACGTACCAGACTACGCAG-3'
Y P Y D V P D Y A

BsiWI

7851: 5'-TCGACTGCGTAGTCTGGTACGTCGTACGGATAC-3'

Sall

EPITOPE: SSEP, 3XEP

8922: 5'-TCGACTATCCGTACGACGTACCAGACTACGCAC-3'

Xhol

8923: 5'-TCGAGTGCAGTAGTCTGGTACGTCGTACGGATAG-3'

Myristylation from c-src 5SMXZ

8908: 5'-CGACACCGCGGCCACCATGGGGAGTAGCAAGAGCAAGCCT
KOZAK M G S S K S K P

SacII

ζ -homology

AAGGACCCCAGCCAGCGCCTCGAGAGGGAGTGCAGAGACTG-3'
K D P S Q R L E R S A E T

SXTZ

Tac

CD3 ζ

homology

Xhol

8912: 5'-CGACACTCGAGGAGCTCTGTGACGATG-3'
E L C D D

Figure 4B/21

Figure 4C/21

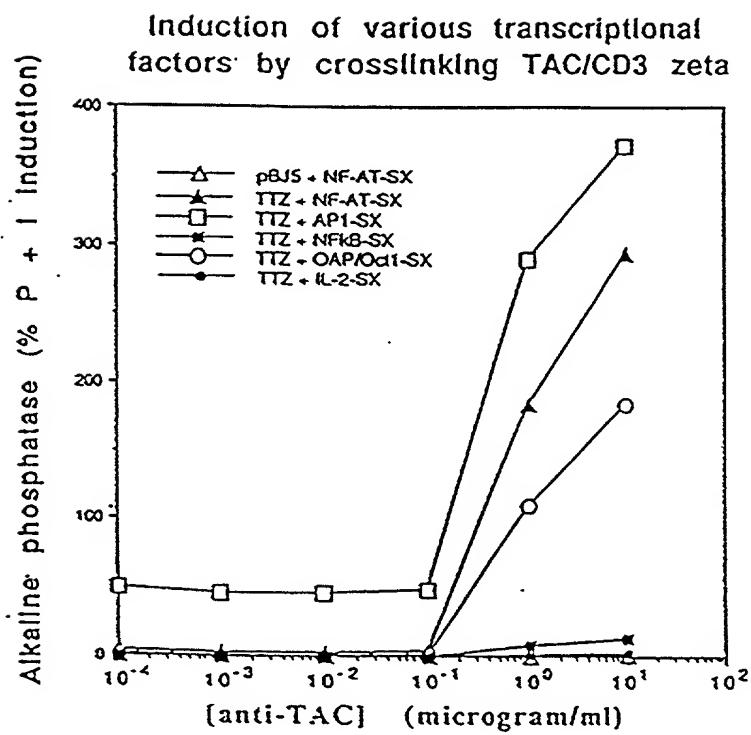


Figure 5/21

Inhibitory activity of dimeric FK506 and CSA

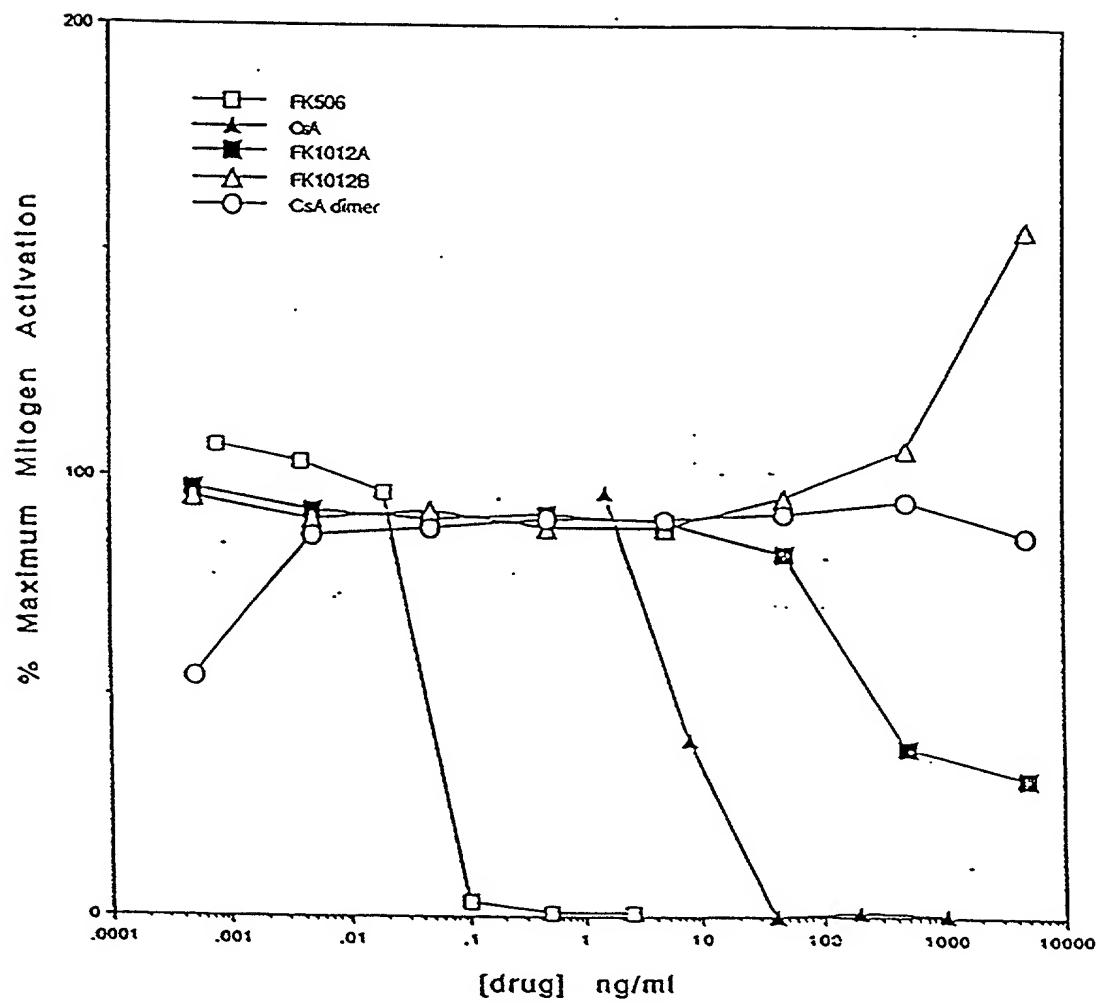
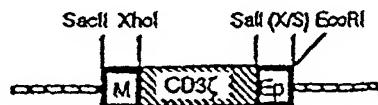
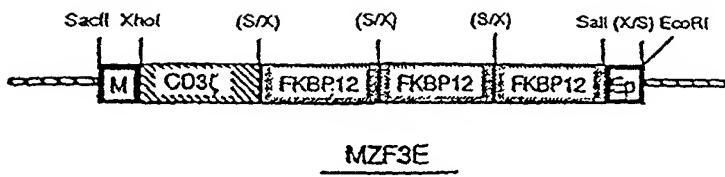


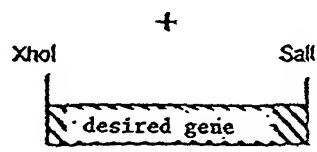
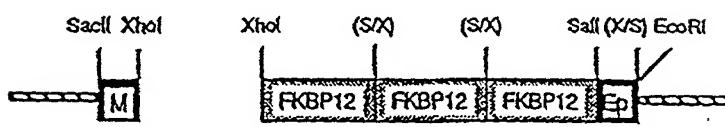
Figure 6A/21



Cut Xhol/SalI; CIP; + FKBP12 X 3



MF3E



- 1. Cytoplasmic moiety of surface receptor
- 2. Tyrosine Kinase
- 3. Transcription Factor
- 4. Others

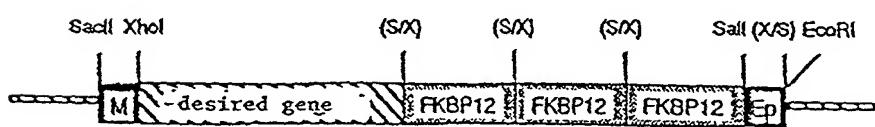


Figure 6B/21

Activity of FK1012A on the chimeric FKBPX3/CD3 zeta receptor

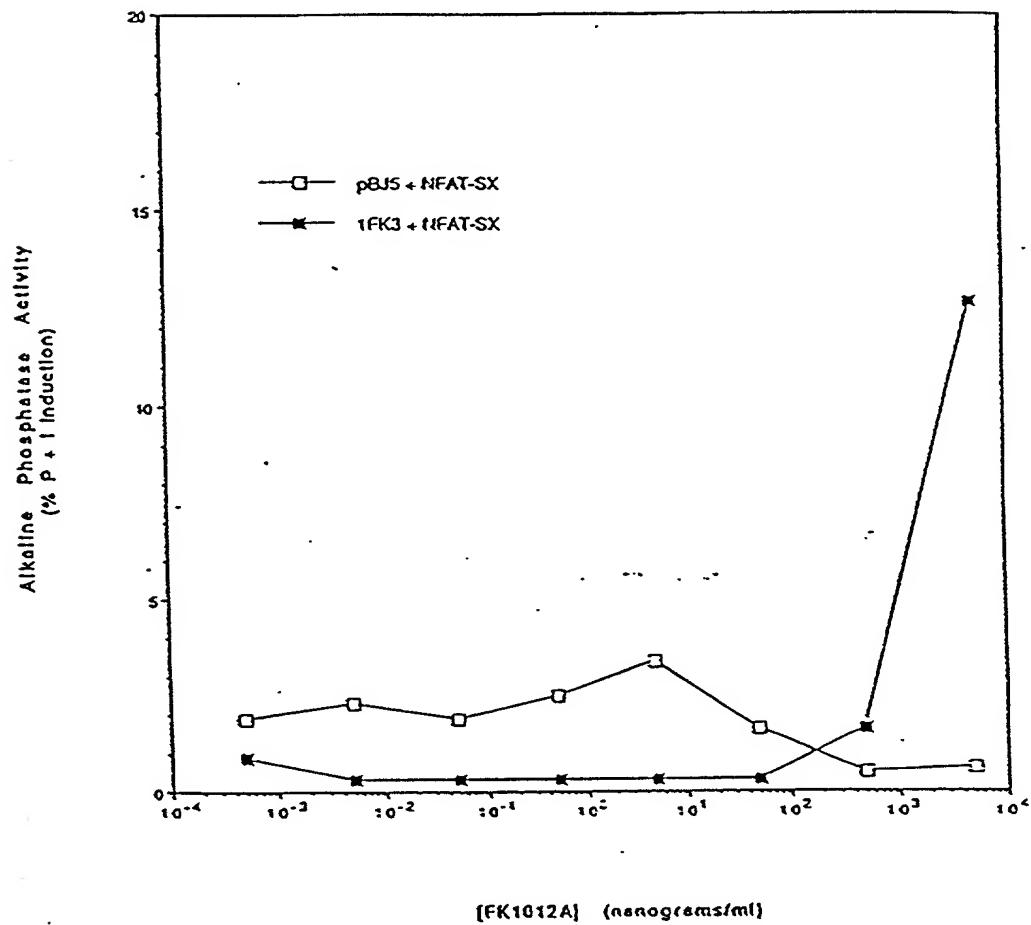


Figure 7/21

Activation of an NFAT reporter via
signalling through a myristoylated
CD3 zeta/FKBP12 chimera

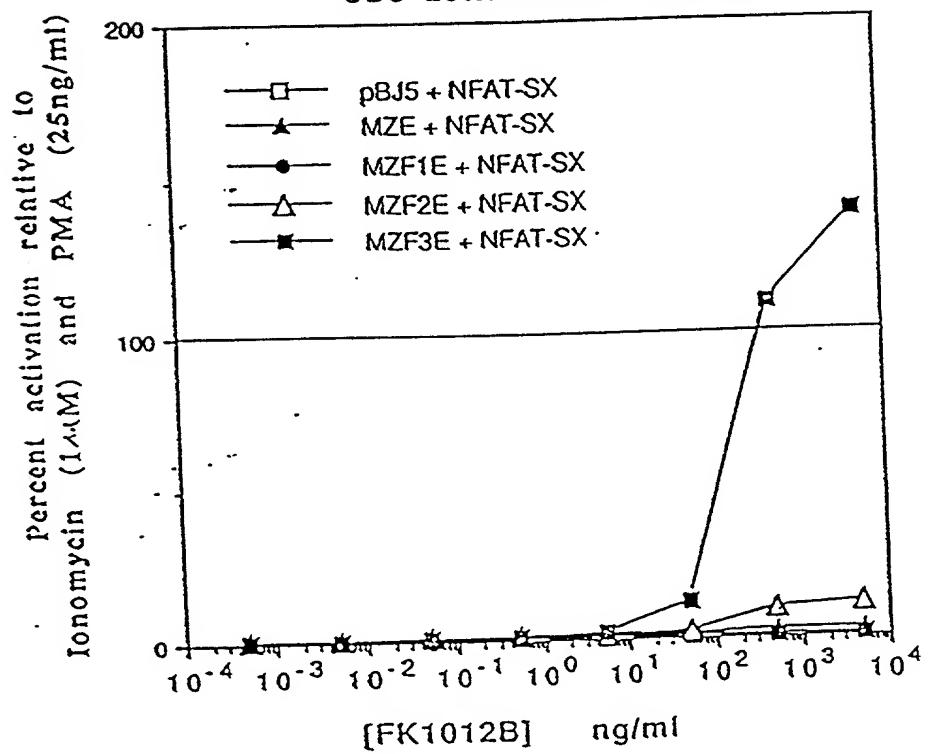


Figure 8/21

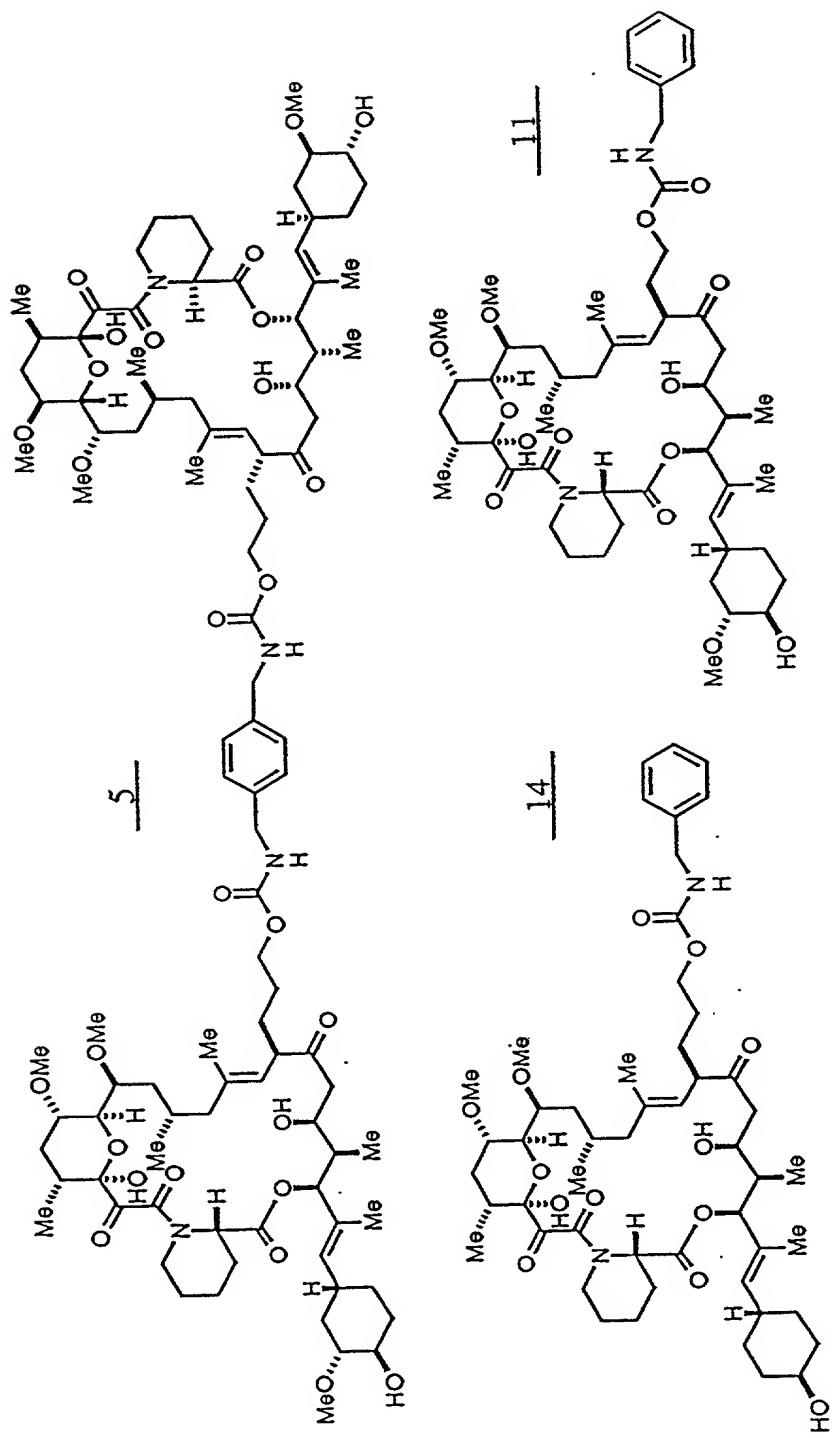


Figure 9A (#1)/21

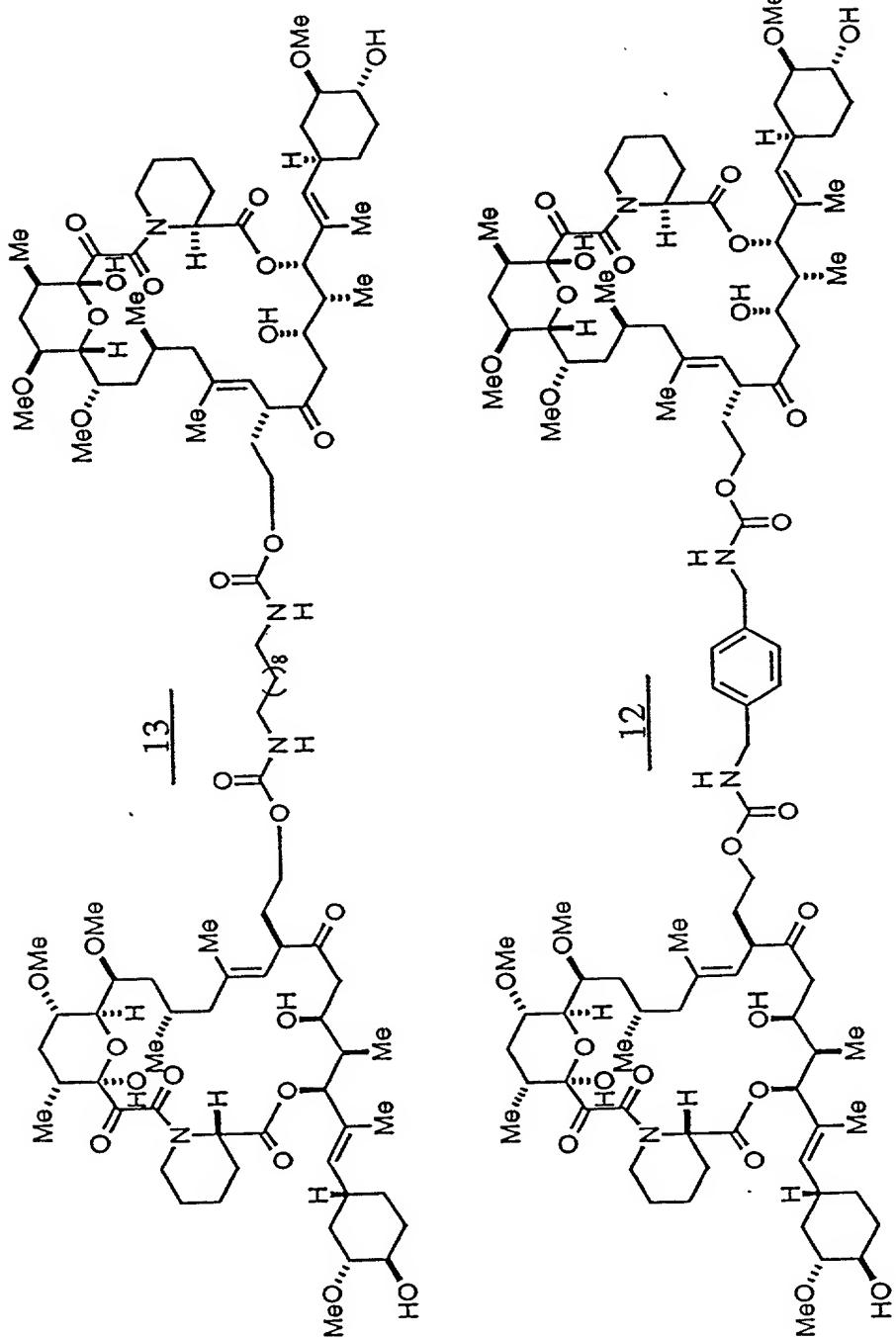


Figure 9A(#2)/2!

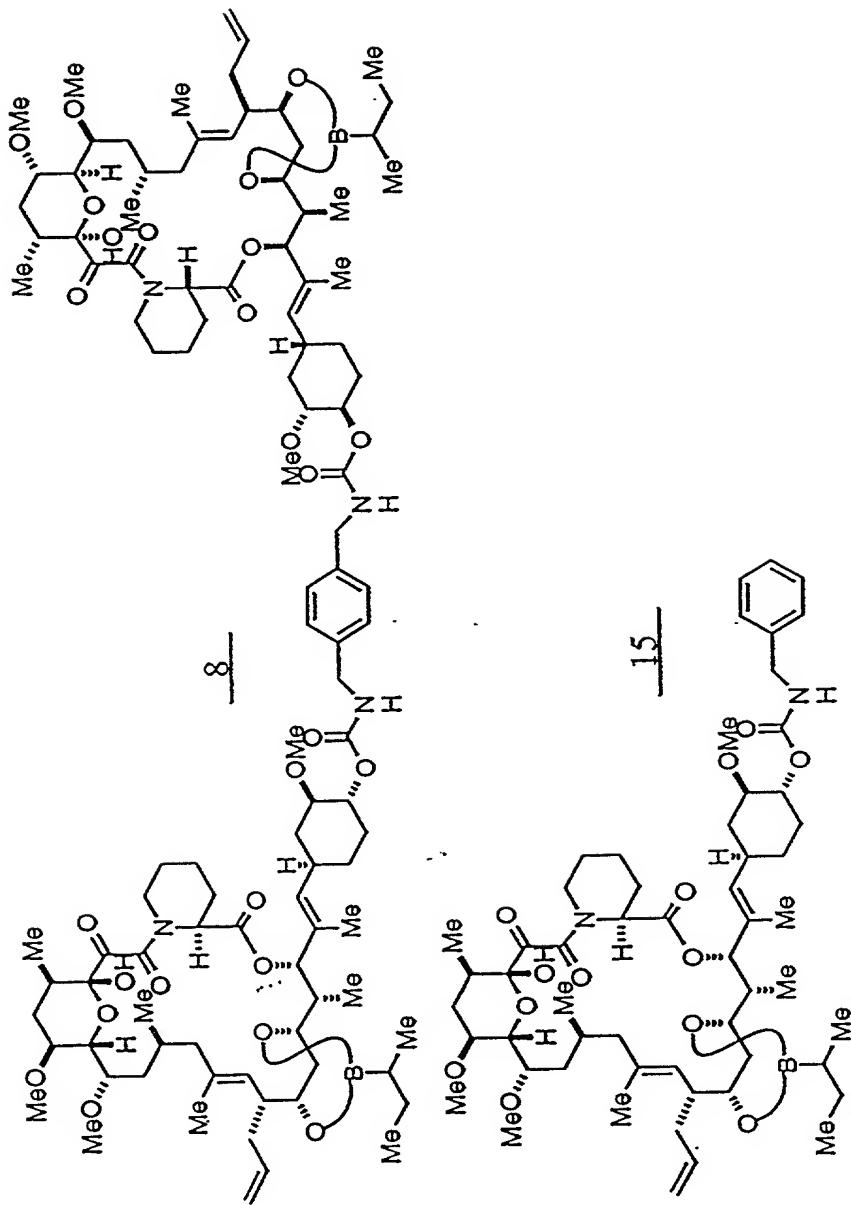


Figure 9B (#1)/21

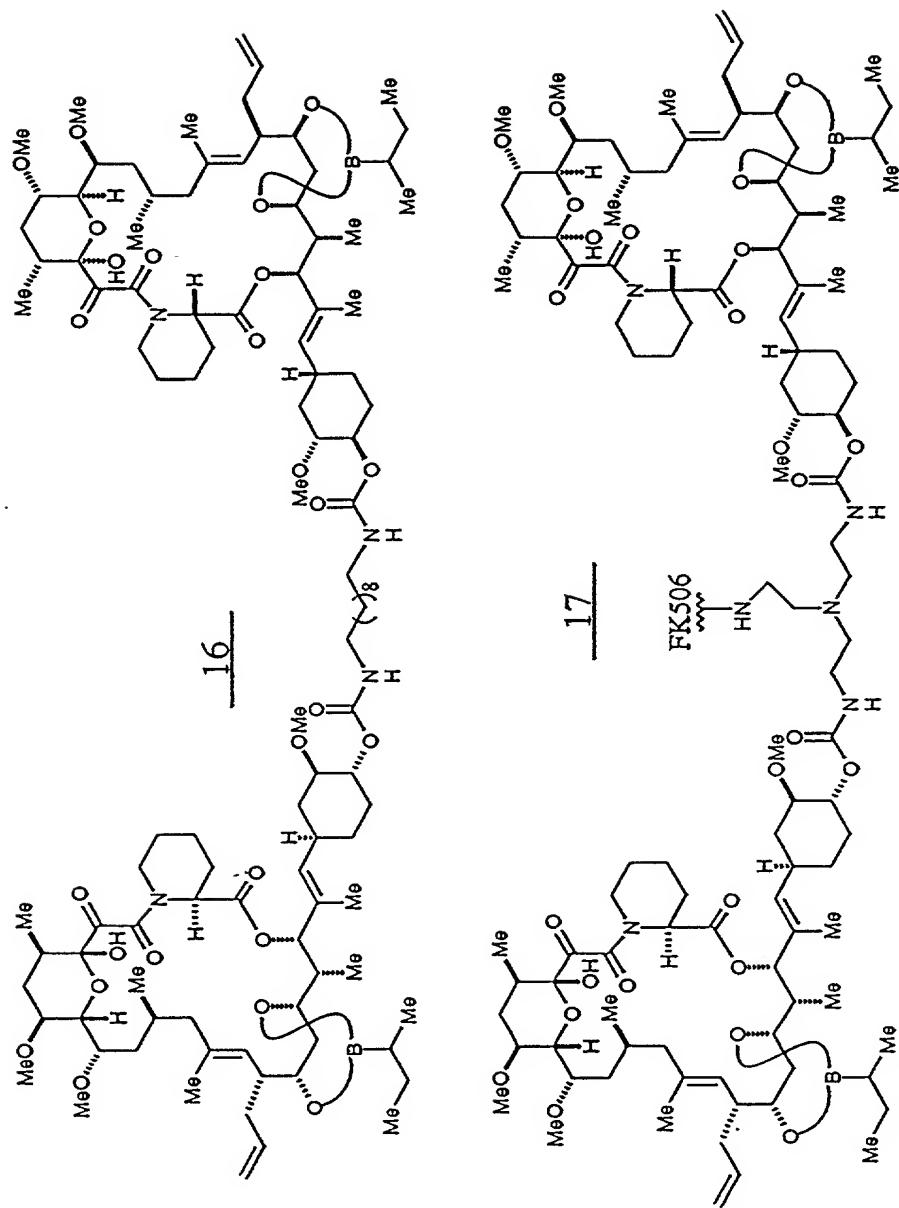
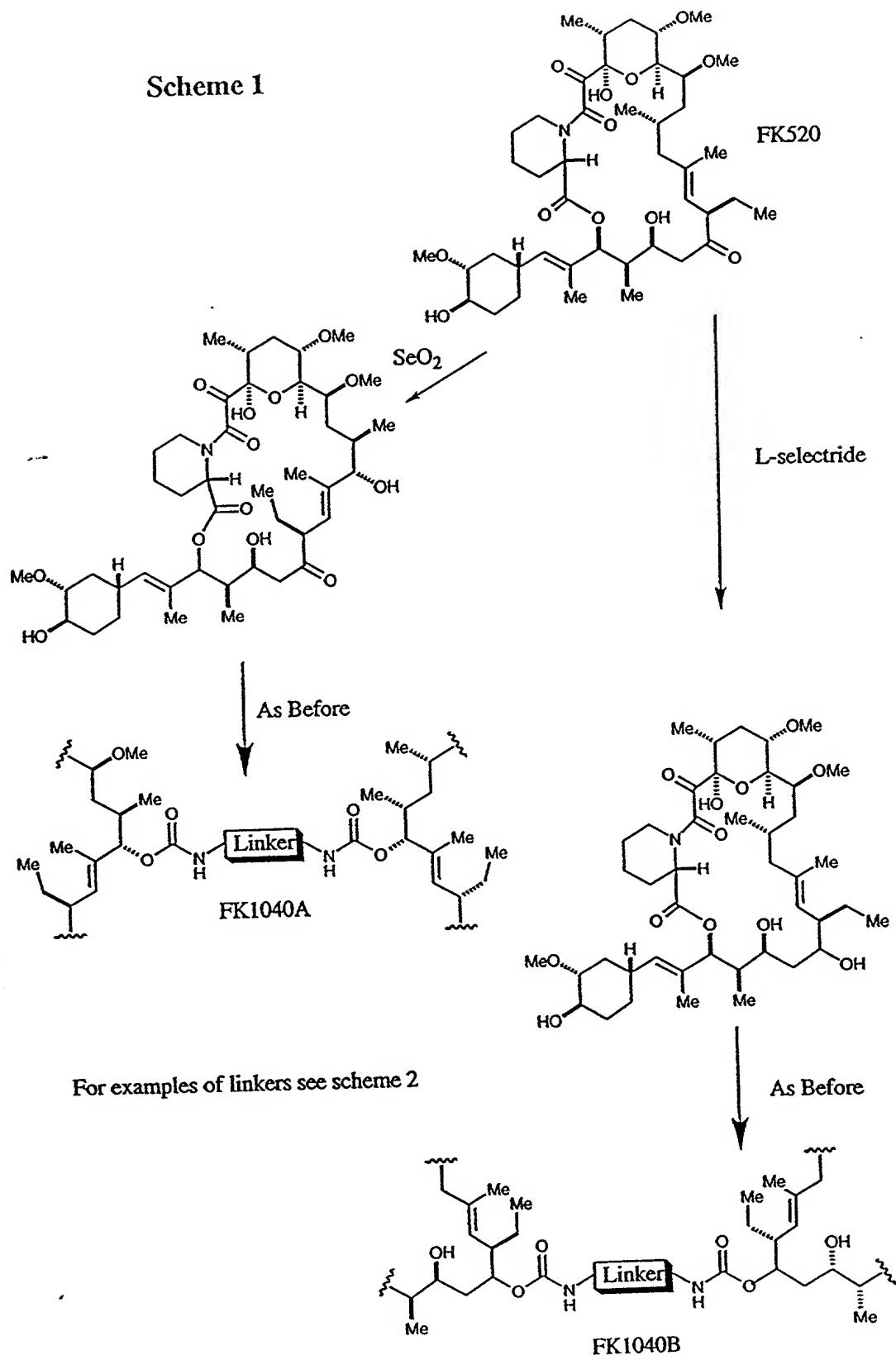


Figure 9B (#2)/21

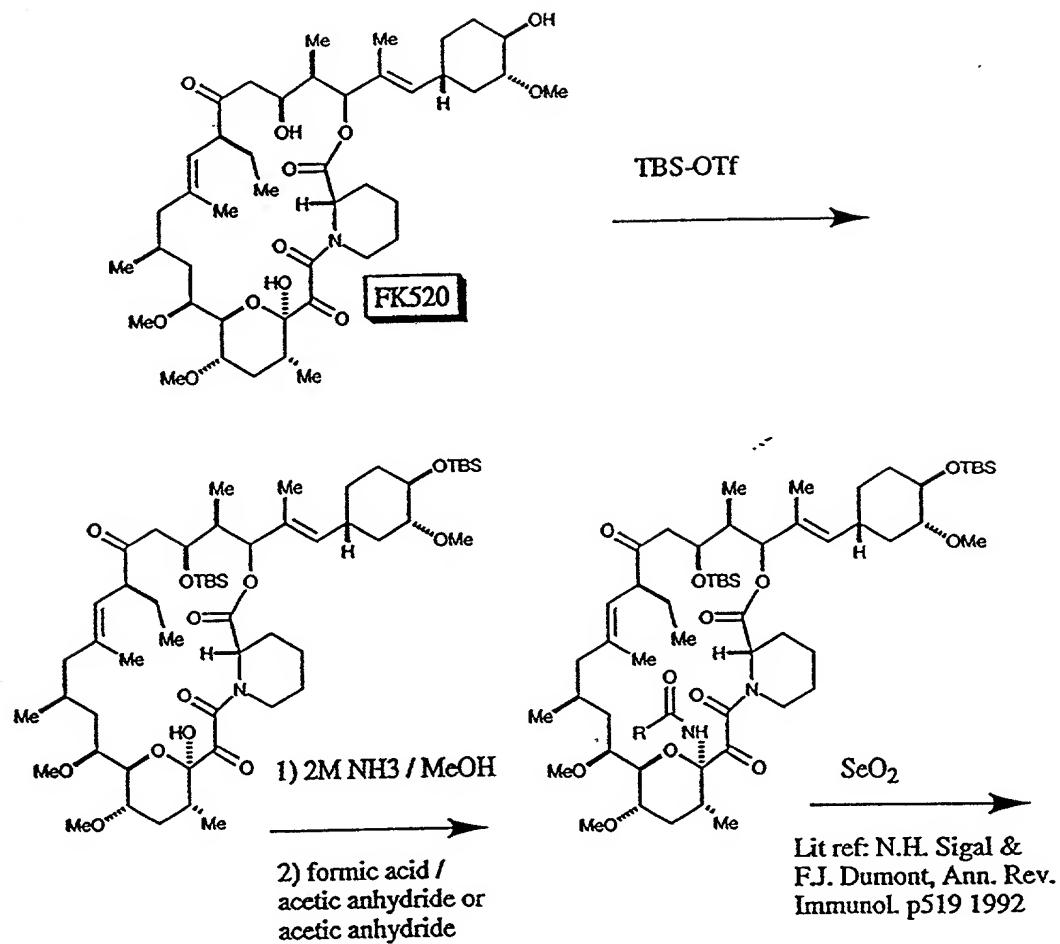
Scheme 1



For examples of linkers see scheme 2

Figure 10/21

Scheme 2: Synthesis of Dimers



Lit refs: D.K. Donald et.al. Tetrahedron Letters p1375, 1991, P.Kocovsky, Tetrahedron Letters p5521, 1992

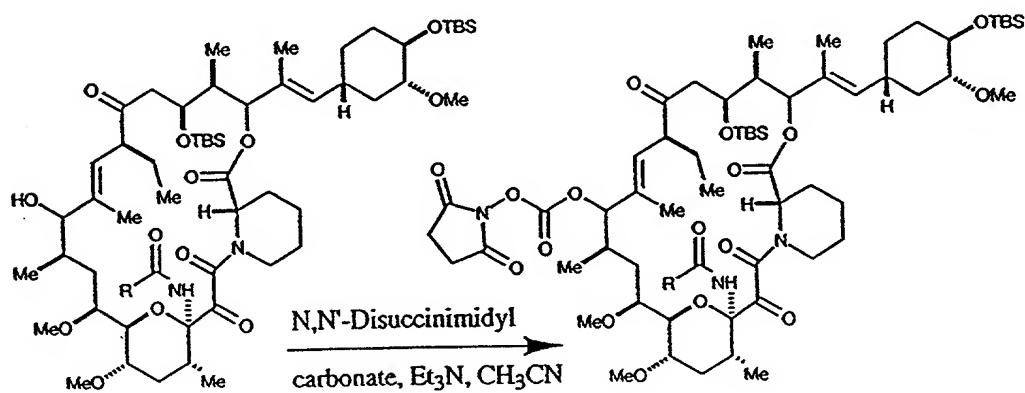


Figure 11A/21

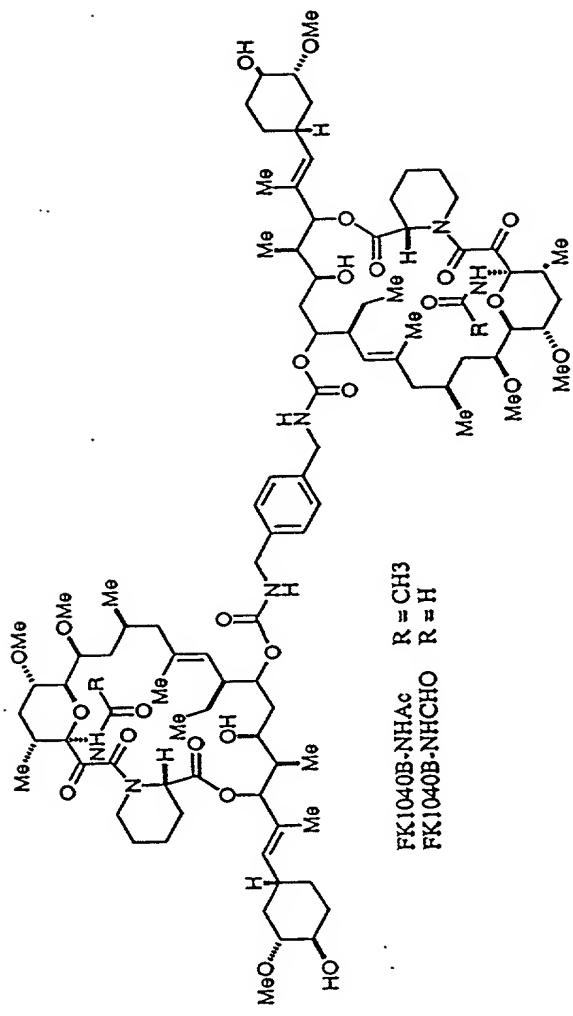
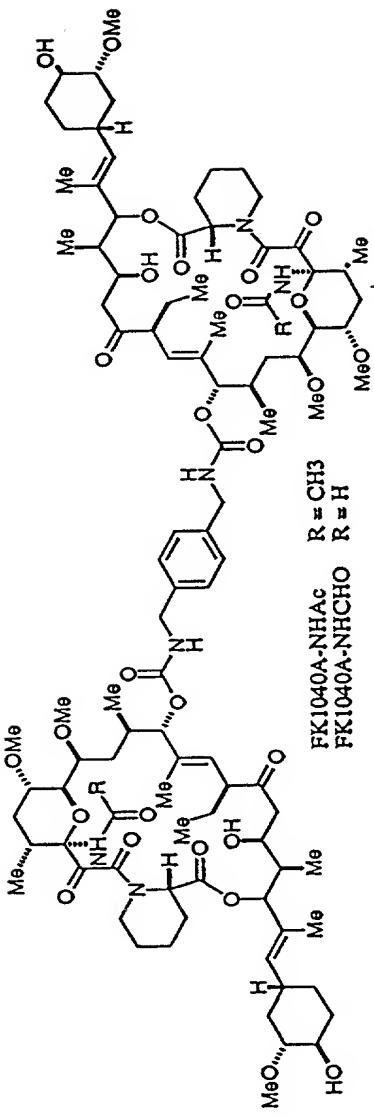


Figure 11B (#1)/21

TCATTTTCAGGCGGTT

An additional modified FK520 (FK1040) that interferes with FKBP12 yet should bind the FKBP12 mutant:
F36A or F99A or Y26A, or combinations thereof is

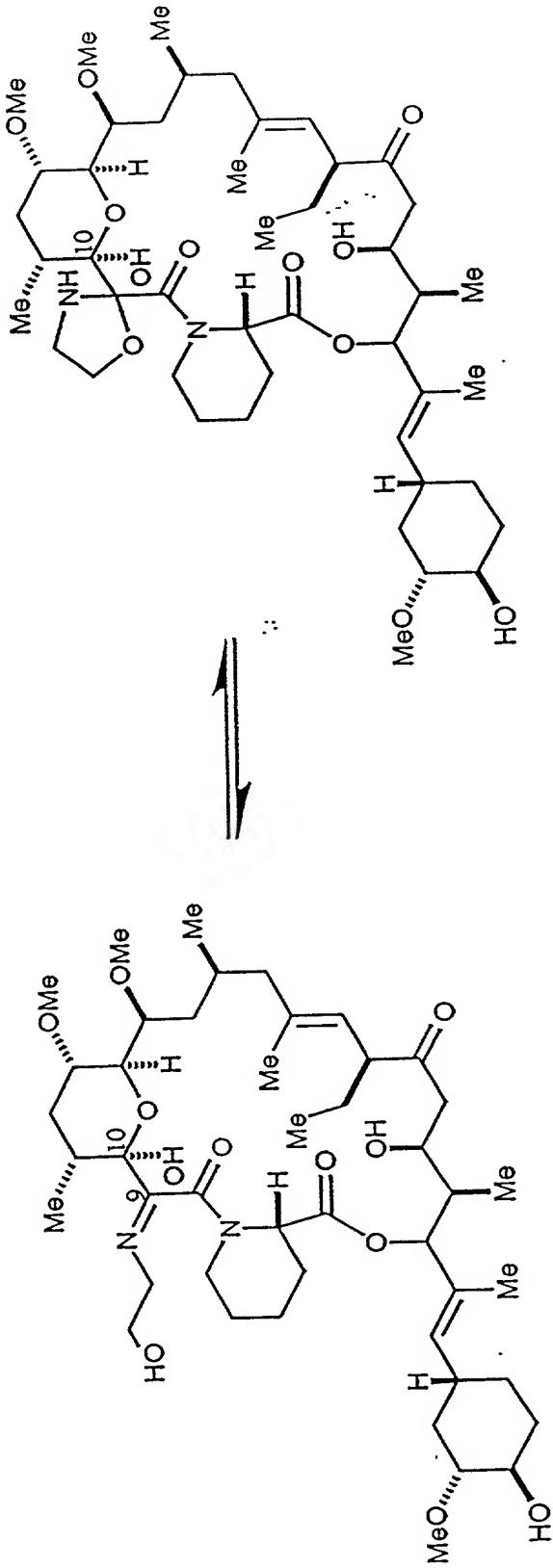


Figure 11B (#2)/21

Scheme 3 Heterodimerization

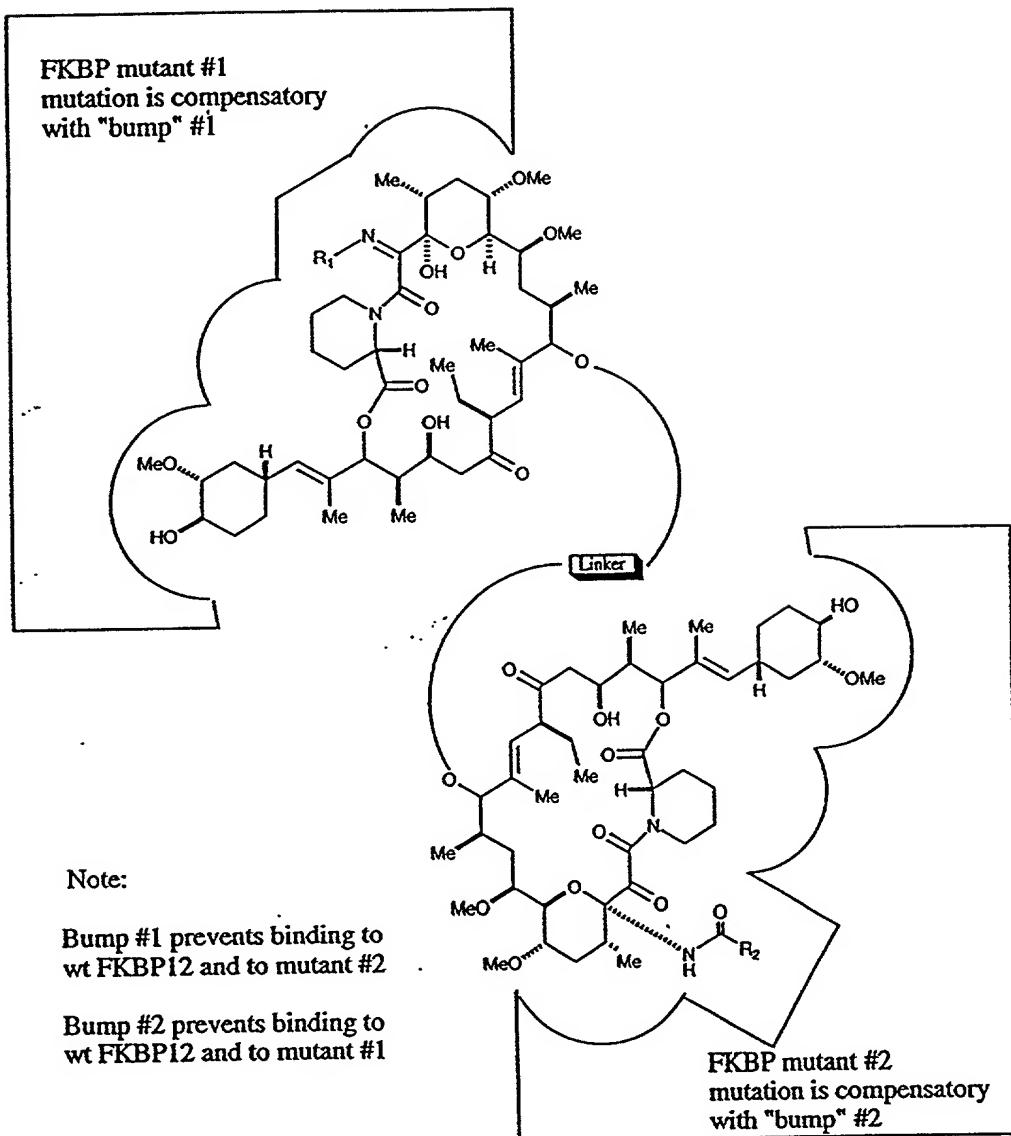
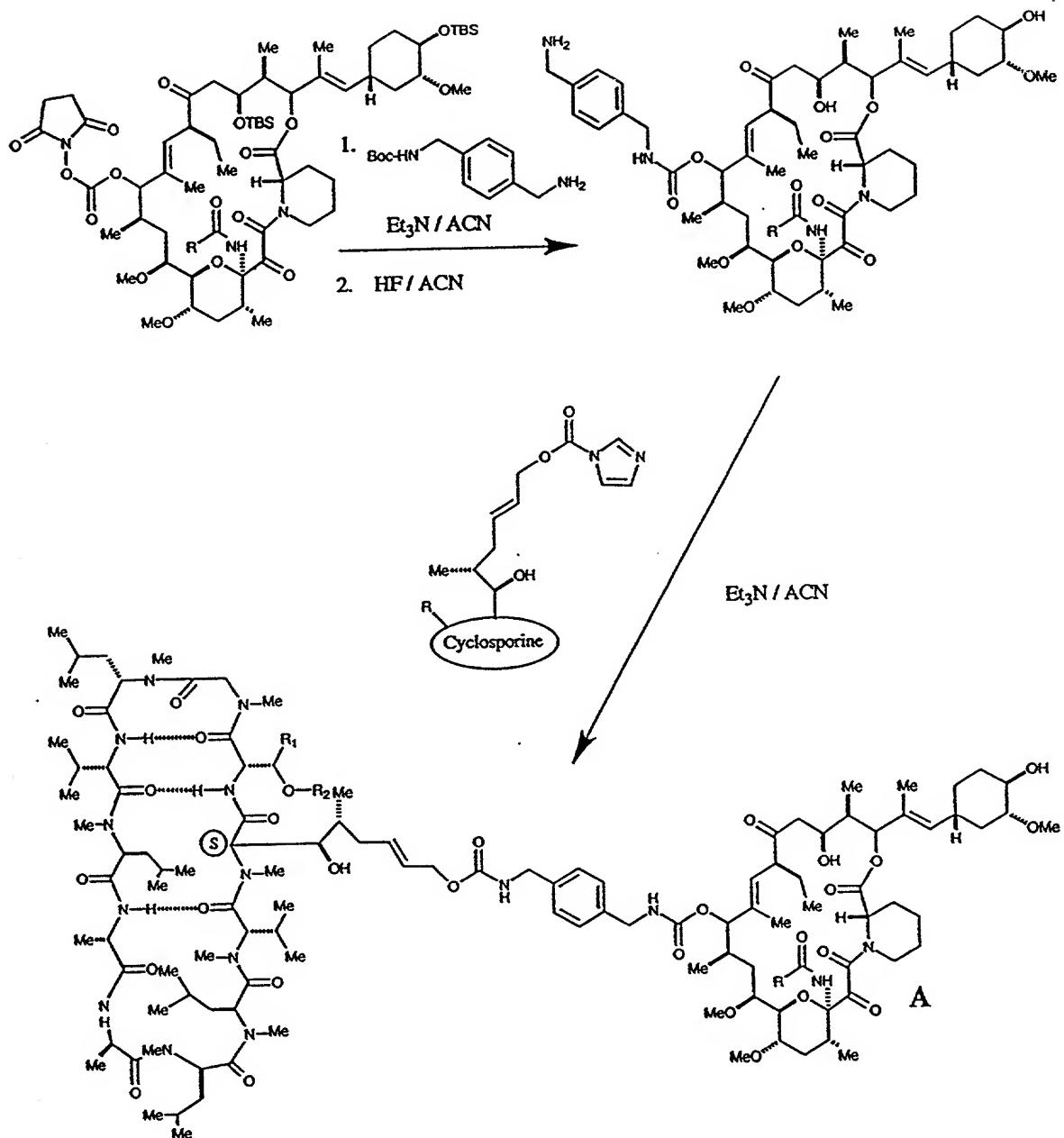


Figure 12/21

Scheme 3: Synthesis of heterodimers



In this example, a heterodimer of a cyclosporine analog and FKS20A-NHCO-R were heterodimerized. However, the scheme can easily incorporate other FK506/S20 derivatives to form hetero or homodimers

Figure 13/21

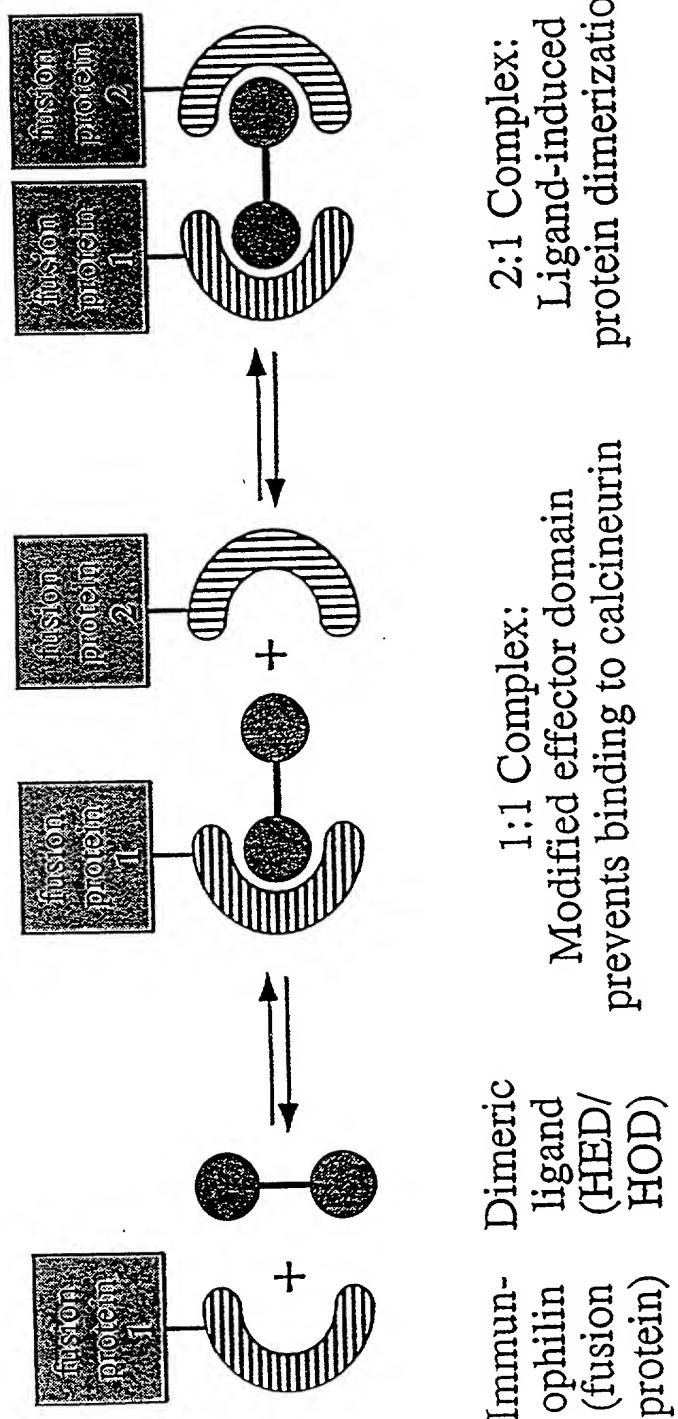


Figure 14/21

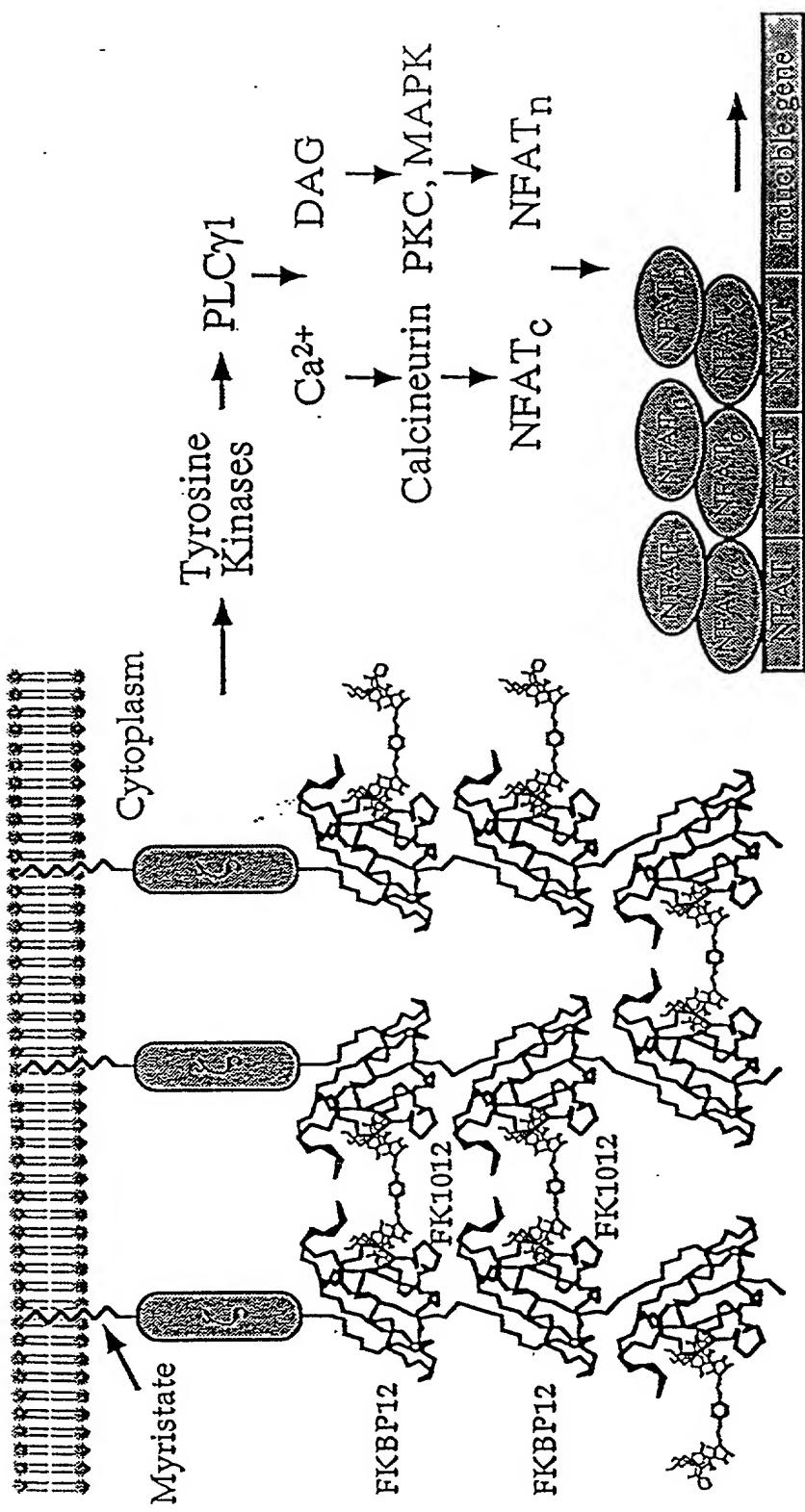
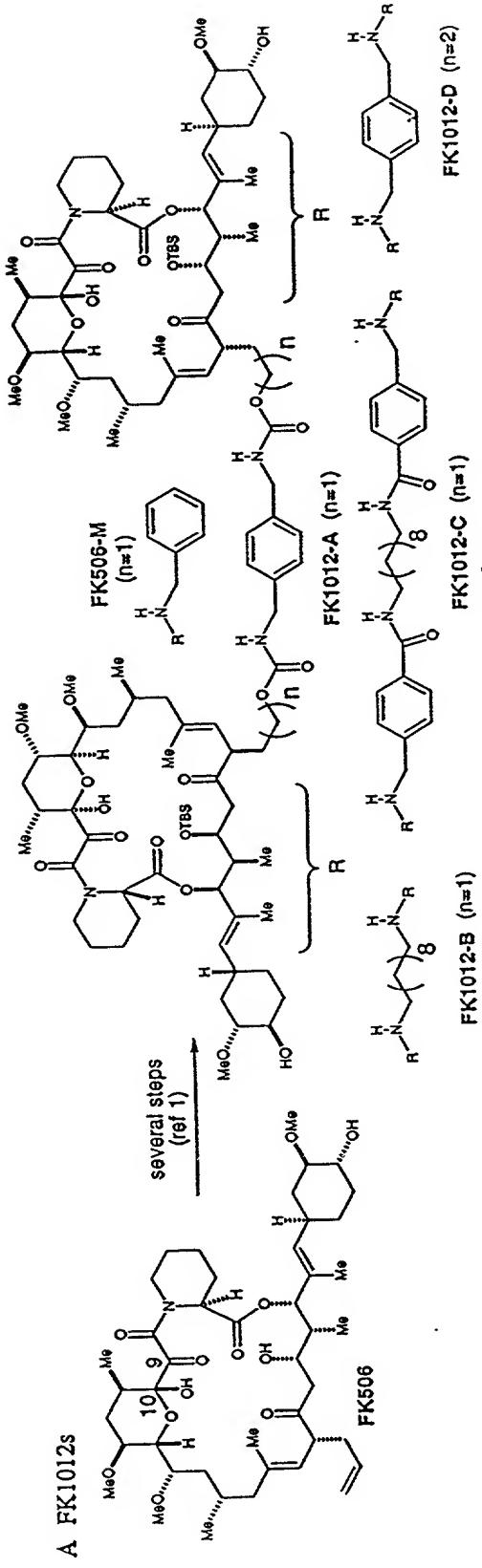


Figure 15/21



B FK506 Monomer with a C10 Bump

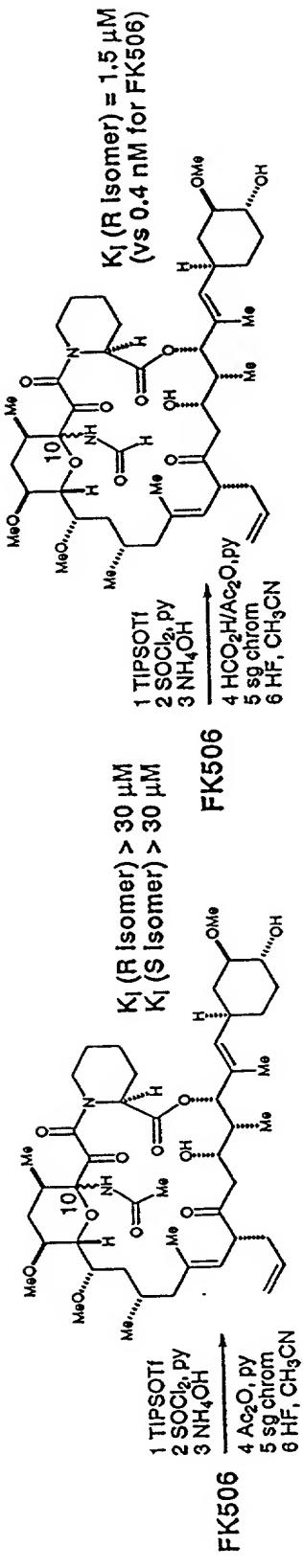
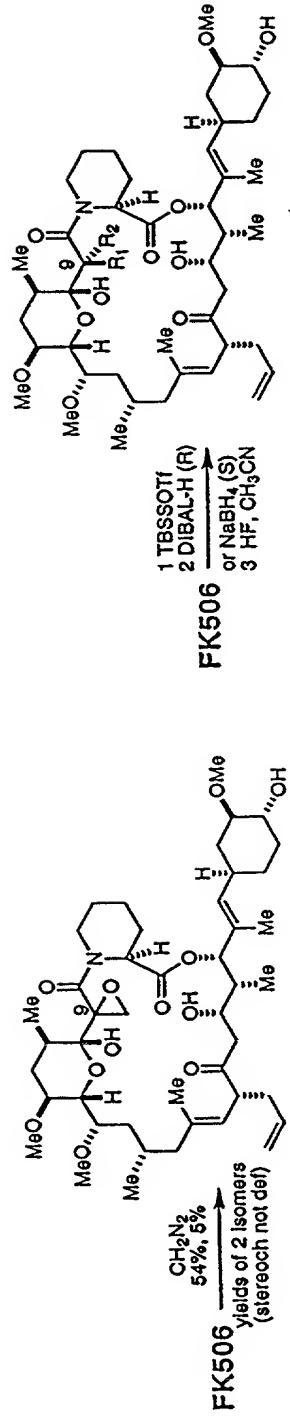


Figure 16 (#1)/21

C FFK506 Monomer with a C9 Bump



D HED Reagent Synthesis

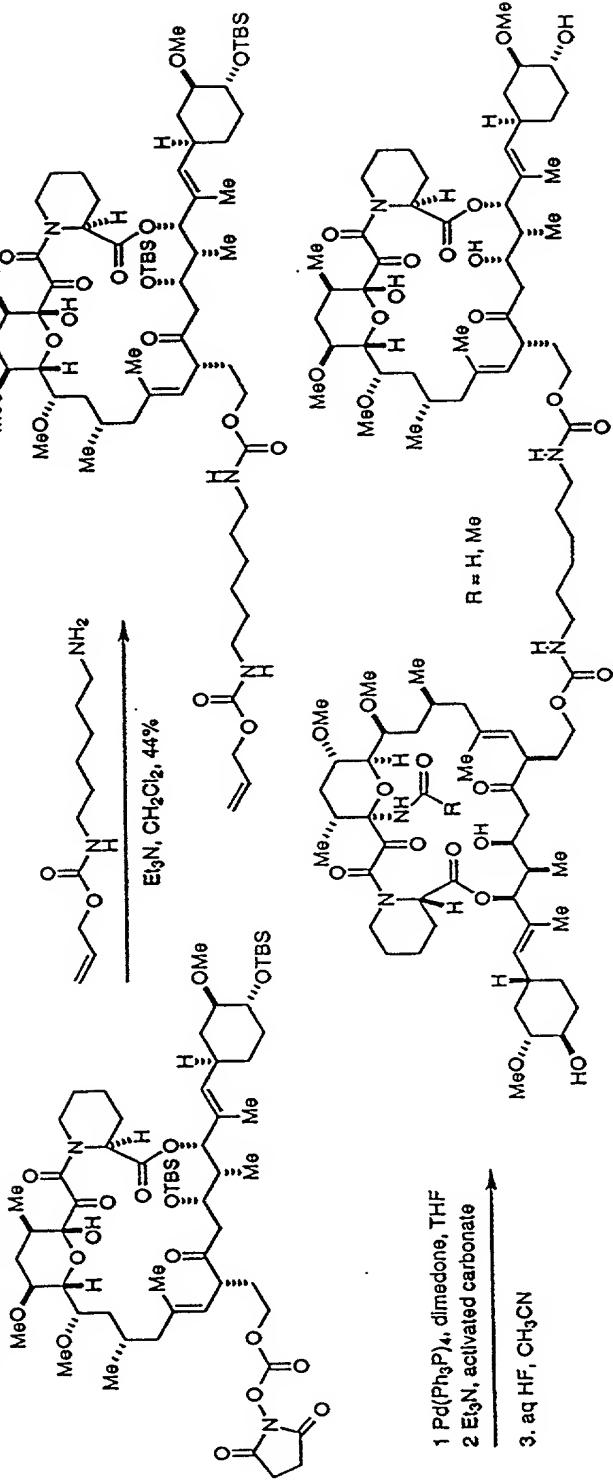


Figure 16 (#2)/21

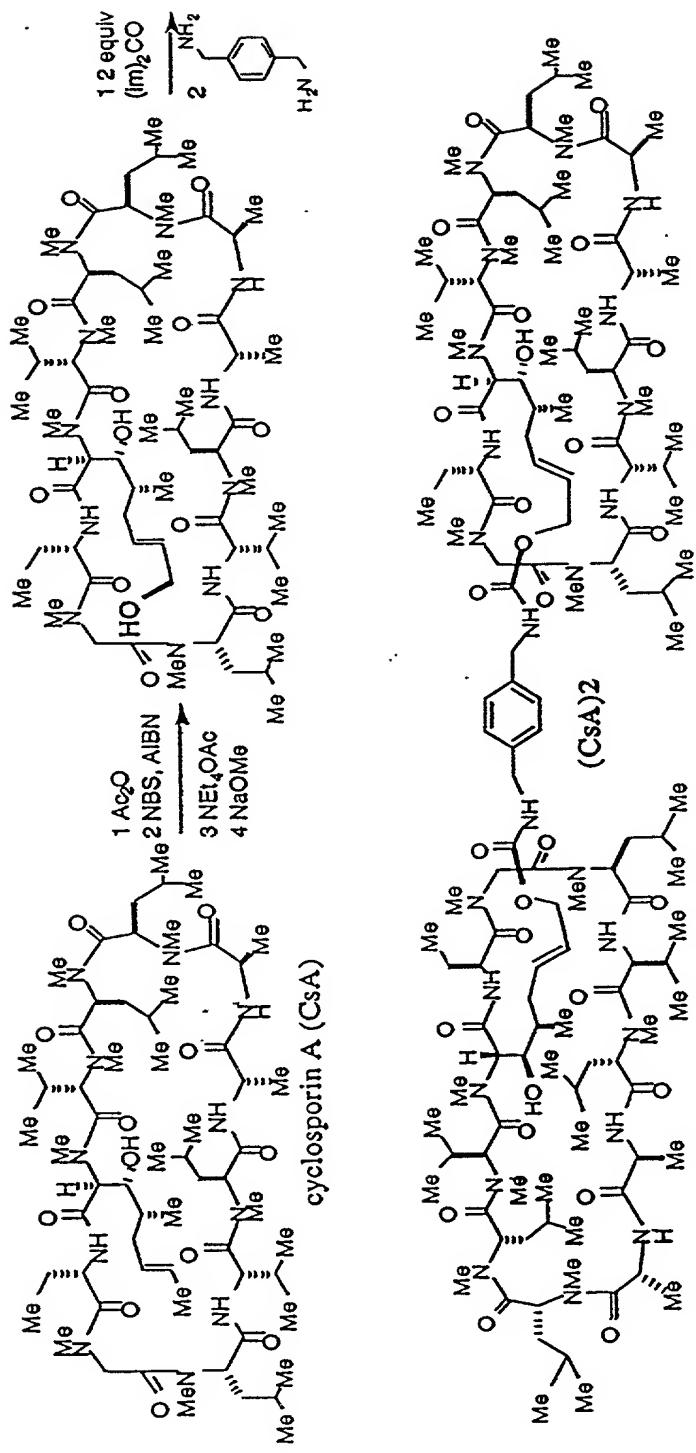
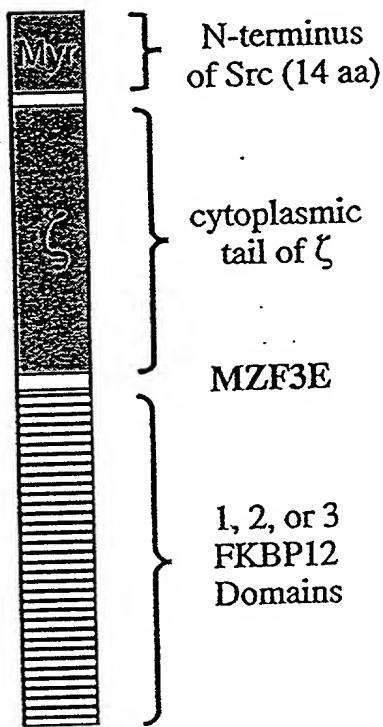


Figure 17/21

A cDNA construct



B expressed protein

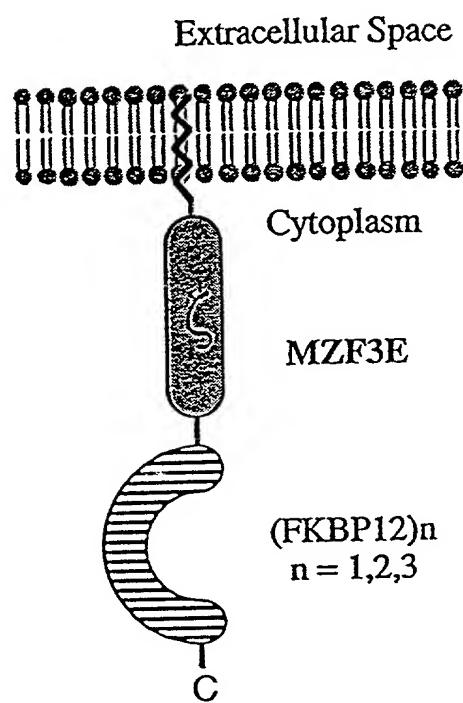


Figure 18A/21

Figure 18B/21

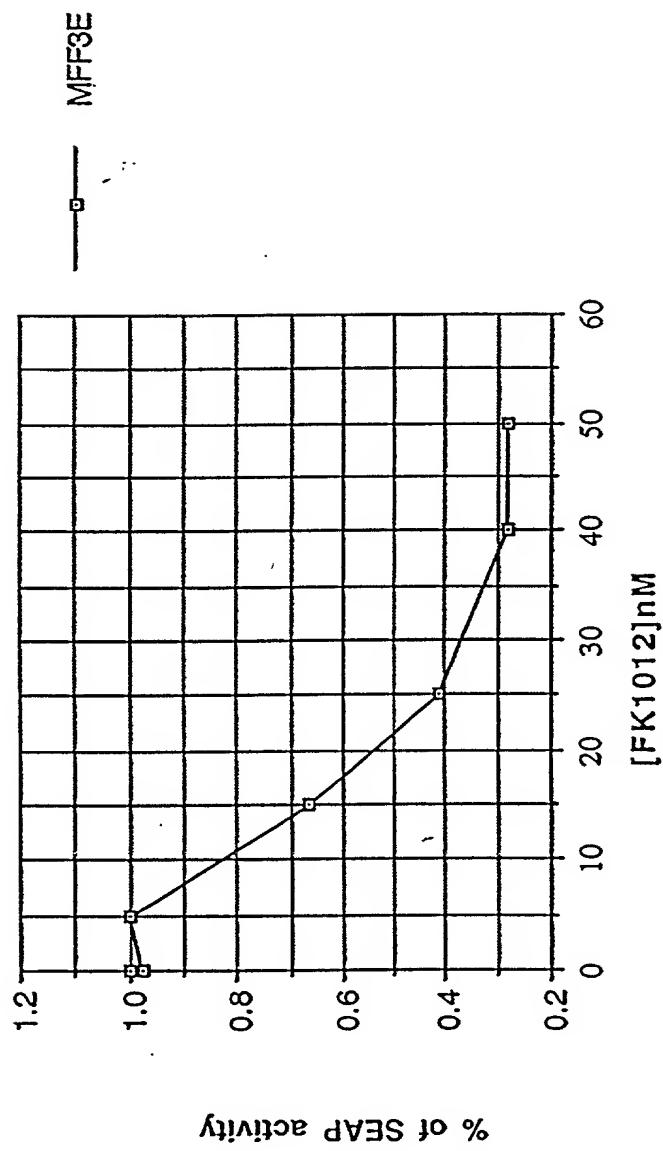


Figure 19/21

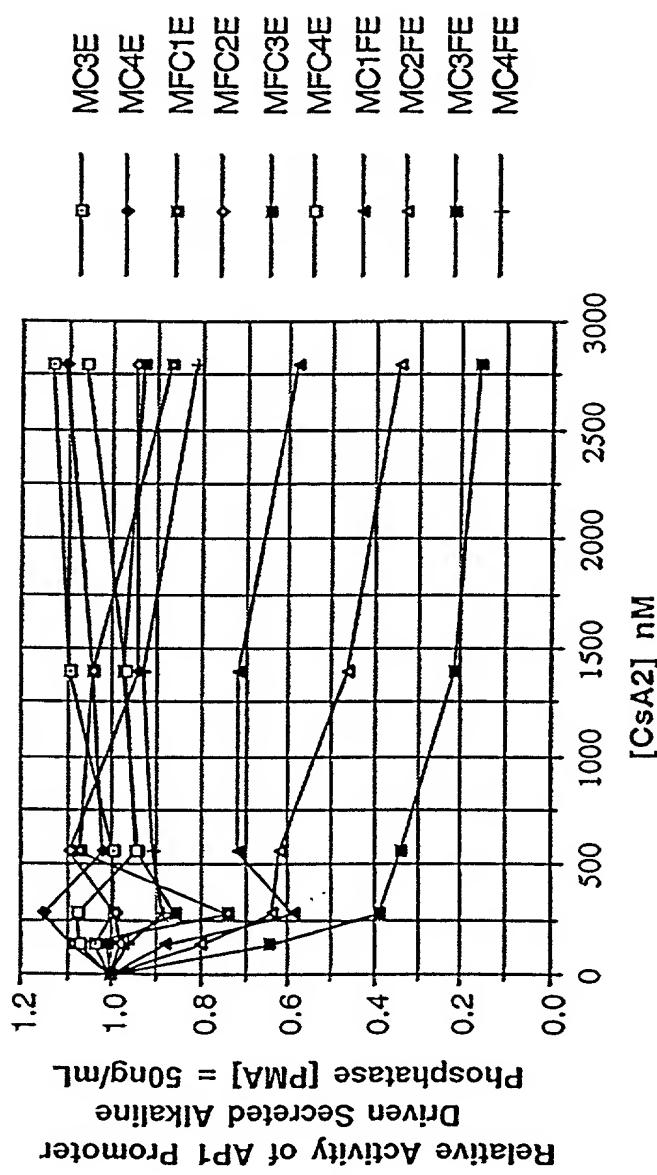
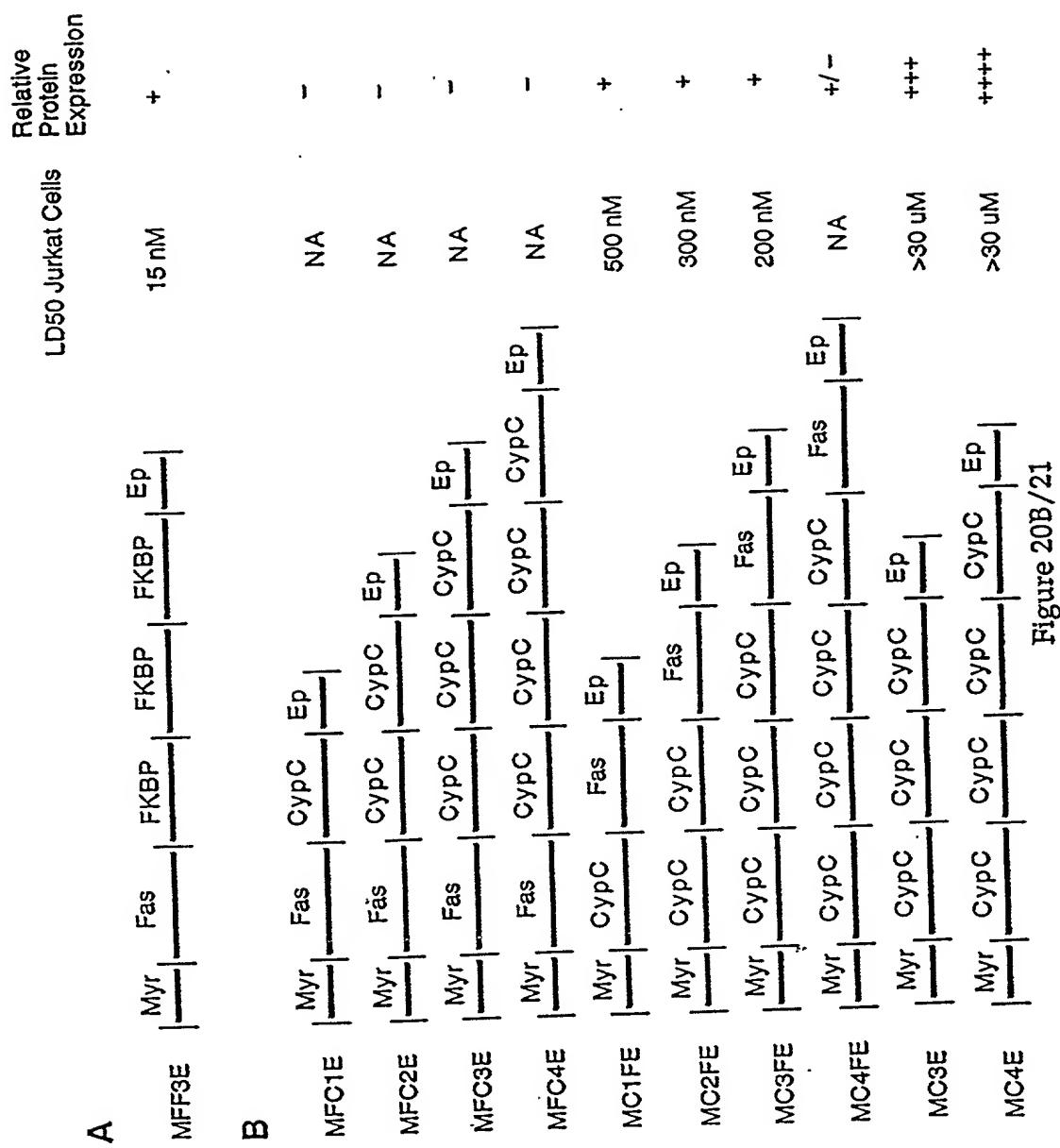


Figure 20A/21



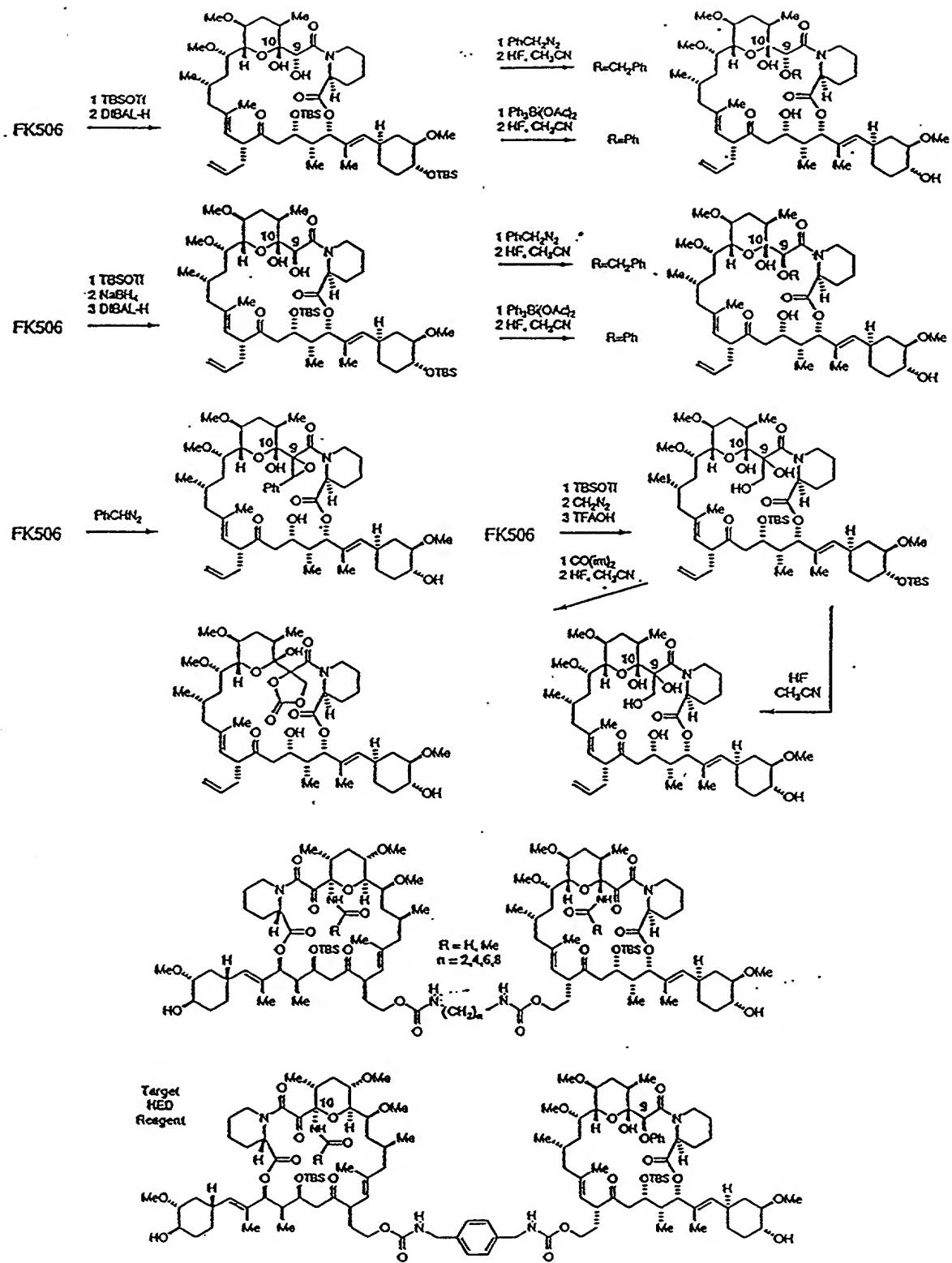


Figure 21/21